(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, moisture, pH, organic chlorine content, free chloride content, crystallinity, and identity.

(ii) Samples required:

(*a*) If the batch is packaged for repacking or for use in the manufacture of another drug:

(*1*) For all tests except sterility: 10 packages, each containing approximately 500 milligrams.

(*2*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(*b*) If the batch is packaged for dispensing:

(*1*) For all tests except sterility: A minimum of 15 immediate containers.

(*2*) For sterility testing: 20 immediate containers, collected at regular intervals throughout each filling operation.

(b) *Tests and methods of assay*—(1) *Potency.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(i) *Sample preparation.* Dissolve an accurately weighed sample in sufficient 1 percent potassium phosphate buffer, pH 6.0 (solution 1), for the microbiological agar diffusion assay and the hydroxylamine colorimetric assay or in distilled water for the iodometric assay, to give a stock solution of convenient concentration; and also if it is packaged for dispensing, reconstitute as directed in the labeling. Then, using a suitable hypodermic needle and syringe, remove all of the withdrawable contents if it is represented as a single-dose container, or if the labeling specifies the amount of potency in a given volume of the resultant preparation, remove an accurately measured representative portion from each container. Dilute with either solution 1 or distilled water, as specified above, to give a stock solution of convenient concentration.

(ii) *Assay procedures.* Use any of the following methods; however, the results obtained from the microbiological agar diffusion assay shall be conclusive.

(a) Microbiological agar diffusion assay. Proceed as directed in § 436.105 of this chapter, diluting an aliquot of the stock solution with solution 1 to the reference concentration of 5 micrograms of dicloxacillin per milliliter (estimated).

(*b*) *Iodometric assay.* Proceed as directed in § 436.204 of this subchapter.

(*c*) *Hydroxylamine colorimetric assay.* Proceed as directed in § 436.205 of this subchapter.

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 20 milligrams of dicloxacillin per milliliter.

(4) [Reserved]

(5) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this subchapter, using an aqueous solution containing 10 milligrams per milliliter (or using a solution reconstituted as directed in the labeling if it is packaged for dispensing).

(7) *Organic chlorine content.* Proceed as directed in § 440.19(b)(5).

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

(9) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 50 FR 19918, May 13, 1985]

§ 440.25 Hetacillin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin is 6-(2,2-Dimethyl-5-oxo-4-phenyl-1-imidazolidinyl)-3,3-dimethyl-7-oxo-4-thia-1-azabicyclo[3.2.0]heptane-2-carboxylic acid. It occurs as a fine, white to off-white powder. It is so purified and dried that:

(i) Its potency is not less than 810 micrograms of ampicillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 2.5 nor more than 5.5.

(v) Its hetacillin content is not less than 90 and not more than 105 percent.

(vi) It gives a positive identity test for hetacillin.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Hetacillin content*—(i) *Reagents*—(*a*) *Hydrochloric acid-acetone solution.* Dilute 8.5 milliliters of concentrated hydrochloric acid to 1 liter with acetone and mix well. Use for 1 day only.

(*b*) *p-Dimethylaminocinnamalde- hyde solution.* Dissolve 0.5 gram of *p*-dimethylaminocinnamaldehyde in sufficient hydrochloric acid-acetone solution to a final volume of 100 milliliters and shake well, filtering if necessary. Prepare immediately before use.

(ii) *Preparation of standard solutions.* Transfer about 100 milligrams of the hetacillin working standard, accurately weighed, to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 0.5, 1.0, and 2.0 milliliters into three respective 25-milliliter volumetric flasks. Add 1.5 and 1.0 milliliters of 0.1*N* hydrochloric acid respectively to the first and second flasks to bring the volume in each to 2.0 milliliters.

(iii) *Blank.* Use 2.0 milliliters of 0.1*N* hydrochloric acid in a 25-milliliter volumetric flask.

(iv) *Preparation of sample solutions.* Using a mortar and pestle, grind the sample to a fine powder. Transfer an accurately weighed portion of about 100 milligrams to a 200-milliliter volumetric flask. Add 150 milliliters of refrigerated distilled water and 20 milliliters of 1*N* hydrochloric acid, shake, dilute to volume with distilled water, and mix well. Transfer 1.0 milliliter to a 25-milliliter volumetric flask, add 1.0 milliliter of 0.1*N* hydrochloric acid, and mix.

(v) *Procedure.* To each of the flasks containing standards, blank, and sample, add 15 milliliters of hydrochloric acid-acetone solution and mix. Then add 3 milliliters of *p*- dimethylaminocinnamaldehyde solution to each and mix. Add 3 milliliters of 0.1*N* hydrochloric acid to each, dilute to volume with hydrochloric acid-acetone solution, mix well, and allow to stand at 25° C. for exactly 30 minutes. (Filter the sample solutions, if necessary, to remove any turbidity.) Using a suitable spectrophotometer, read the absorbance values of standard and sample solutions at a wavelength of 515 nanometers against the blank. Plot the absorbance values of the standards versus their concentrations and read the sample concentration from this standard response line.

(vi) *Calculations.*

$$\text{Percent hetacillin} = \frac{C \times 5{,}000 \times P}{\text{Weight of sample in milligrams}}$$

where:

C=Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

P=Hetacillin content of the hetacillin working standard in percent.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 44 FR 10379, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.29 Hetacillin potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality and purity.* Hetacillin potassium is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram.

(ii) [Reserved]

(iii) Its moisture content is not more than 1.0 percent.

(iv) Its pH in an aqueous solution containing 10 milligrams per milliliter is not less than 7.0 and not more than 9.0.

(v) Its hetacillin content is not less than 82 percent and not more than 95.5 percent.

(vi) It gives a positive identity test for hetacillin potassium.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, moisture, pH, hetacillin content, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed for ampicillin in § 436.105 of this chapter, using the ampicillin working standard as the standard of comparison and preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute the stock solution with solution 3 to the reference concentration of 0.1 microgram of ampicillin per milliliter (estimated).

(2) [Reserved]

(3) *Moisture.* Proceed as directed in § 436.201 of this chapter.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using an aqueous solution containing 10 milligrams per milliliter.

(5) *Hetacillin content.* Proceed as directed in § 440.25(b)(5), except use about 110 milligrams of sample and calculate the hetacillin content as follows:

$$\text{Percent hetacillin} = \frac{C \times 5{,}000 \times P}{\text{Weight of sample in milligrams}}$$

where:

C=Concentration in milligrams of hetacillin per milliliter of the final solution of the sample obtained from the standard response line.

P=Hetacillin content of the hetacillin working standard in percent.

(6) *Identity.* Proceed as directed in § 436.211 of this chapter, using a 1 percent potassium bromide disc prepared as directed in paragraph (b)(1) of that section.

(7) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 18976, May 30, 1974, as amended at 42 FR 59857, Nov. 22, 1977; 44 FR 10379, Feb. 20, 1979; 50 FR 19918, May 13, 1985]

§ 440.29a Sterile hetacillin potassium.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Hetacillin potassium is the potassium salt of hetacillin. It occurs as a fine, white to light buff powder. It is so purified and dried that:

(i) Its potency is not less than 735 micrograms of ampicillin per milligram. If it is packaged for dispensing, its potency is satisfactory if it contains not less than 90 percent and not more than 120 percent of the number of milligrams of ampicillin that it is represented to contain.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its moisture content is not more than 1.0 percent.